

Application Note

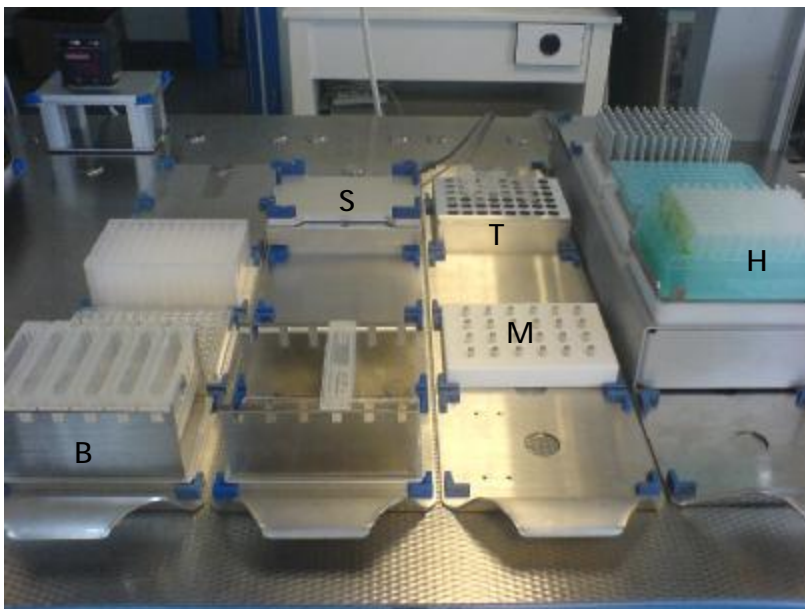
Fully automated genomic DNA extraction from wheat with CyBi®-RoboSpense using MACHEREY-NAGEL NucleoMag 96 Plant Kit

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Abstract: The MACHEREY-NAGEL NucleoMag 96 Plant kit technology for purification of genomic DNA from plant tissue has been automated on the CyBi®-RoboSpense. The robotic microplate handler, electronically temperature controlled unit and shaker for microplates were used to perform a fully automated procedure. The resulting DNA samples are extracted with very good yields (10µg DNA from 80mg wheat leaves) and excellent purity (OD 260nm/280nm=1.7).

Introduction: This purification method is based on reversible adsorption of nucleic acids to paramagnetic beads under appropriate buffer conditions. After mechanical disruption, plant tissue is extracted with CTAB-Lysis Buffer. Binding conditions are adjusted and the NucleoMag C-Beads are added to the samples. After magnetic separation and removal of supernatant, the paramagnetic beads are washed four times. Finally, highly purified DNA is eluted with Elution Buffer.



- B buffers
- S shaker
- T microreaction tube rack
- M NucleoMag SEP
- H electronically temperature controlled unit

Figure 1: Process layout for automation of the NucleoMag 96 Plant Kit

Methods: The MACHEREY-NAGEL NucleoMag 96 Plant kit (1x96 preps, MN Cat. No. 744 400.1) extraction protocol was set up on a CyBi®-RoboSpense system consisting of a 35 position deck equipped with 8 liquid handling channels and disposable tips, 8 high precision pumps with a volume range from 1–1000µL, a temperature controlled position, a microplate shaker, a microplate handler and MACHEREY-NAGEL's NucleoMag SEP separator.

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The purification procedure was established according to MACHEREY-NAGEL's standard protocol for NucleoMag 96 Plant kit. The protocol was carried out with 80mg freshly homogenized wheat leaves (*Triticum aestivum*) per well.

The quality and integrity of the DNA obtained was analysed by gel electrophoresis and ethidium bromide staining. Yields were calculated by DNA quantification with SYBR Green I.

Purity of extracted DNA was determined by measurement of OD at 260nm and 280nm with a SpectraMax 250 microplate reader and by calculating the $A_{260/280}$ ratio. For an additional quality check, PCR amplification of a gene fragment of NADH-dehydrogenase (831bp) was performed with 35 PCR cycles.

Automated extraction procedure:

1. Place reagents, tips, samples, and buffers on the CyBi[®]-RoboSpense deck as shown in Figure 1.
2. Manually homogenize fresh wheat leaves in Lysis Buffer (10 leaves in 500 μ L) in a Dispomix[®] (BioLab Products GmbH) for 7.5min at 2500rpm.
3. Add 400 μ L Lysis Buffer MC1 including RNase to the homogenization tubes containing 80mg plant tissue. Shake for 30sec at 750rpm and incubate for 30min at 65°C.
4. Optional step: Manually clear lysate by transferring samples to centrifuge for 20min at 5,000xg
5. Transfer 400 μ L of cleared lysate to a Square-well Block with the CyBi[®]-RoboSpense
6. Add 400 μ L Binding Buffer MC2 and 30 μ L C-Beads. Shake 5min at 1000rpm.
7. Move the Square-well Block to the NucleoMag SEP and wait for 1min. Remove supernatant.
8. Wash sample by adding 600 μ L Wash Buffer MC3 and shake for 5min. Separate the beads for 1min. Remove Wash Buffer.
9. Wash sample in 600 μ L Wash Buffer MC4, shake for 5min and separate beads for 1min. Remove Wash Buffer.
10. Add 600 μ L of 80% ethanol, shake for 5min, separate for 1 min and remove liquid.
11. Wash sample in 600 μ L Wash Buffer MC5, incubate for 1min, aspirate and discard supernatant
12. Elute purified sample into the elution plate by adding 200 μ L Elution Buffer MC6, shake 5min, incubate for 5min at 55°C and separate for 2min. Transfer the supernatant into the elution plate.

Results: Purified genomic DNA demonstrated high quality and was successfully used for a downstream PCR amplification (Figure 2). DNA extraction yield was calculated at 10 μ g from 80mg plant (n=16). Purity was determined by measurement of OD 260nm/280nm and calculated to be 1.7.

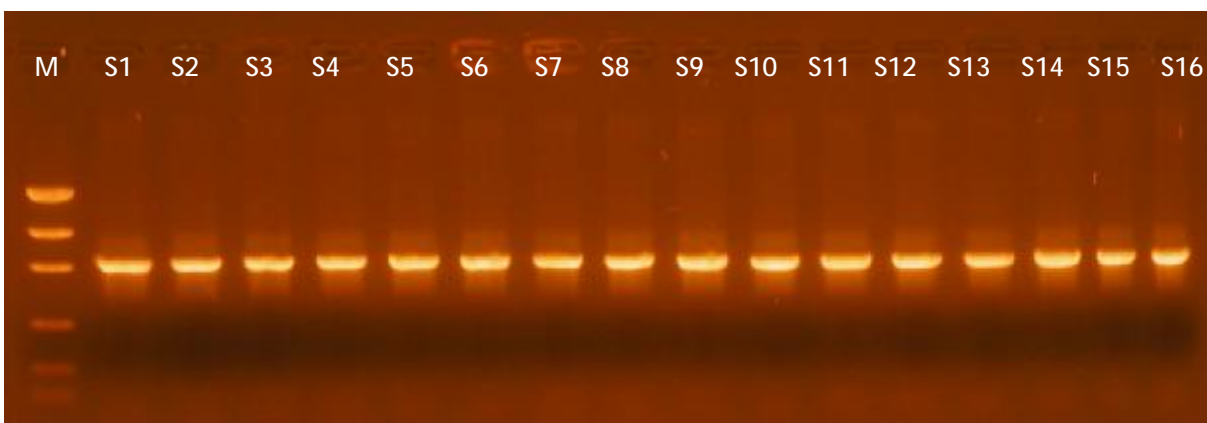


Figure 2: PCR amplification of NADH-dehydrogenase gene fragment (831bp). Analysis of extracted DNA for their usability in the downstream application PCR. A 831bp long NADH- dehydrogenase gene fragment was amplified by PCR with 35 cycles and analyzed by gel electrophoresis. Lane M: 4 μ L Low DNA Mass Ladder (Invitrogen); lanes S1 -S16 : 10 μ L of PCR reaction.

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Discussion: The special accessory modules, such as the shaker for microplates and the temperature controlled unit, made it possible to implement the automated assay on the CyBi®-RoboSpense with excellent results.

Making use of the individually addressable pipetting probes, the C-Beads were aspirated from a single microreaction tube and distributed across the plate with a multi dispense mode.

By shaking the Square-well microplate at 1000rpm on CyBio's shaker for microplates, full resuspension of C-Beads could be achieved. This resulted in a decrease of process time and tip consumption because resuspension by pipetting up and down after adding binding, wash or elution buffer wasn't necessary.

Magnetic bead separation time on NucleoMag SEP was optimized to one minute to avoid the irreversible formation of a clump of beads that could not be pipetted or dispersed. Previous tests have shown that a full separation of beads and supernatant was reached already after one minute.

Complete removal of residual buffers during the separation steps in order to avoid decreased binding efficiency from remaining buffer was achieved by optimization of the liquid handling parameters.

Careful adjustment of pipetting speed, implementation of pre- and post-delays and air gaps ensured safe handling of the samples and the different buffers with unique physical properties.

Precise adjustment of tip heights for aspirating or dispensing in the wells of the processed plate helped to avoid contact with the bead pellet and thus carry-over of beads was eliminated.

DNA was eluted in 200µL elution buffer to assure full resuspension of all beads and increase the elution yields.

Optimization of pipetting parameters such as aspirate and dispense heights, delays, and air gaps, and optimization of bead separation times allowed maximum yields from the automated procedure.

The results clearly demonstrate that the CyBi®-RoboSpense is optimally suited for reliable automation of genomic DNA extraction from plant tissue using MACHEREY-NAGEL's NucleoMag 96 technology.